

Amendments to the Specification:

Please replace title in the amended specification dated January 8 2004 with the following original title:

Manufacturing processes for ~~Se-methyl-L-selenocysteine~~ Se-alkylselenocysteine, Se-allylselenocysteine, Se-arylselenocysteine.

Please replace paragraph [0004] in the amended specification dated January 8 2004 with the following original paragraph:

[0004] The present patent describes efficient processes for the manufacture of L-Se-methylselenocysteine, D-Se-methylselenocysteine and DL-Se-methylselenocysteine: The structures of the referred materials are shown below; Using the same chemical process, manufacture of Se-alkylselenocysteine, Se-allylselenocysteine, Se-arylselenocysteine is possible.

Please delete paragraphs [0007.1] and [0007.2] appearing after paragraph [0007] on page 3 of the amended specification dated January 8 2004 .

Please replace paragraph [0011] in the amended specification dated January 8 2004 with the following original paragraph:

[0011] L-Chloroalanine methyl ester hydrochloride (IIa) was synthesized by a convenient method from the reaction of L-Serine methyl ester hydrochloride with ~~phosphorous pentachloride in chloroform solution. The method of Walsh~~ thionyl chloride in a solvent. The methods described earlier in the literature are not very convenient to use. For example, L-Serine methyl ester hydrochloride was reacted with phosphorous pentachloride in chloroform solution to give chloroalanine methyl ester hydrochloride (Walsh, C. T.; Schonbrunn, A.; Abeles, R. H.; J Biol Chem., 1971,246 (22), 6855-6866) is but one way of synthesizing L-Chloroalanine methyl ester hydrochloride (IIa). Alternatively other methods could be used. It is difficult to handle highly hygroscopic phosphorous pentachloride. The method described in the present patent uses more easily handled thionyl chloride. Then IIa is converted to L-Chloroalanine hydrochloride (IIb) by reaction with aqueous hydrochloric acid. L-Chloroalanine hydrochloride (IIb) could be neutralized with triethyl amine to form L-Chloroalanine (IIc). As mentioned, IIa, IIb and IIC ~~were~~ are all convenient raw materials for L-Se-methyl selenocysteine.

Please replace paragraph [0013] in the amended specification dated January 8 2004 with the following original paragraph:

[0013] We also found that hypophosphorous acid could be used to cleave Se-Se- bond of dimethyldiselenide and the sodium salt of methylselenol was formed using sodium hydroxide. The methylselenide sodium thus generated was reacted with L-chloroalanine methyl ester hydrochloride (IIa), or L-chloroalanine hydrochloride (IIb) or L-chloroalanine (IIc) to get L-Se-methylselenocysteine. In extension of the above concept, one can use a dialkyldiselenide as a starting material to generate alkylselenide anion which can react with IIa, IIb or IIC to yield L-Se-alkylselenocysteine. Similarly starting with diallyldiselenide and generating allyl selenide anion and further reacting with IIa or with IIb or with IIC, one can obtain L-Se-allylselenocysteine. These are straightforward extensions of the process patented in this application. Likewise diaryldiselenides could be used to generate arylselenol or arylselenide salts which could be used to produce Se-aryl selenocysteine. In an analogous way, D-Se-methylselenocysteine (Ib) is obtained from D-Chloroalanine methyl ester hydrochloride (IIIa) or from D-Chloroalanine hydrochloride (IIIb) or from D-Chloroalanine (IIIc). These raw materials IIIa, IIIb and IIIc are obtainable from D-Serine methyl ester hydrochloride in a similar way described for the L-analogs.

Please replace paragraph [0014] in the amended specification dated January 8 2004 with the following original paragraph:

[0014] By similar processes, one can produce other D-Se-alkylselenocysteine or D-Se-allylselenocysteine or D-Se-aryl selenocysteine. Similarly in an analogous way DL-Se-methylselenocysteine (Ic) is obtained from DL-Chloroalanine methyl ester hydrochloride (IVa) or from DL-Chloroalanine hydrochloride (IVb) or from DL-Chloroalanine (IVc). These raw materials are obtainable from DL-Serine methyl ester hydrochloride as described for the L-analogs.

Please replace paragraph [0015] in the amended specification dated January 8 2004 with the following original paragraph:

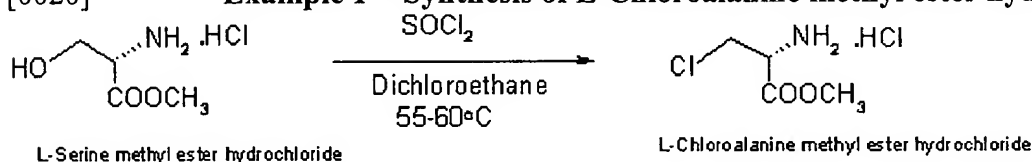
[0015] Extensions of the described processes to manufacture DL-Se-alkylselenocysteine or DL-Se-allylselenocysteine or DL-arylselenocysteine are possible.

Please replace paragraph [0016] in the amended specification dated January 8 2004 with the following original paragraph:

[0016] Additionally DL-Se-methylselenocysteine (Ic) is also obtainable from L-Se-methylselenocysteine (Ia) or from D-Se-methylselenocysteine (Ib) by racemization as the Example 7 ~~Example 4~~ described later in this embodiment will illustrate.

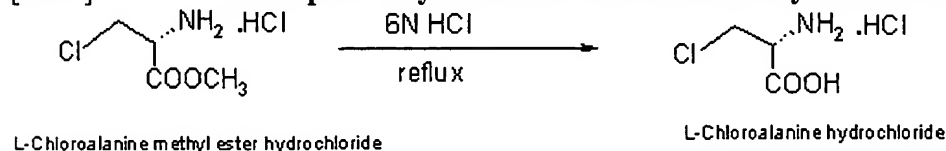
Please retain paragraphs [0020], [0021], and [0022] presented in the original specification.

[0020] **Example 1 - Synthesis of L-Chloroalanine methyl ester hydrochloride:**



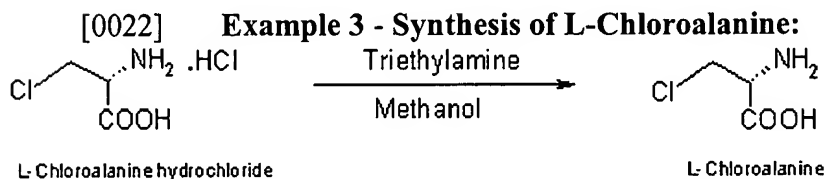
A 500ml RB flask was charged with L-Serine methyl ester hydrochloride (20g, 129 mmol) and dichloroethane and the resulting suspension was heated to 55°C with stirring. Thionyl chloride (30.6g, 258 mmol) was added drop wise, with vigorous stirring of the mixture. The colorless suspension turned into thick white gel during 30 minutes which became a clear yellow solution after next 30-40 minutes. Pale yellow solid started coming out of the clear solution within 10-15 minutes, ultimately making the reaction mixture as thick solid which was kept at 55°C for further 3 hours. The reaction mixture was cooled, transferred to a Buchner flask/funnel set up and using (water) vacuum all the solvent was sucked off to obtain a dry slightly yellow colored solid, which was taken to next step directly without further purification. Yield: 22g (crude weight); Melting point: 153-155°C (dec.); TLC analysis: n-Butanol:Water:Acetic acid (6:2:2); Proton NMR of the product was satisfactory.

[0021] **Example 2 - Synthesis of Chloroalanine hydrochloride:**



L-Chloroalanine methyl ester hydrochloride (21 g) was dissolved in 6N hydrochloric acid and refluxed for 4 hours.

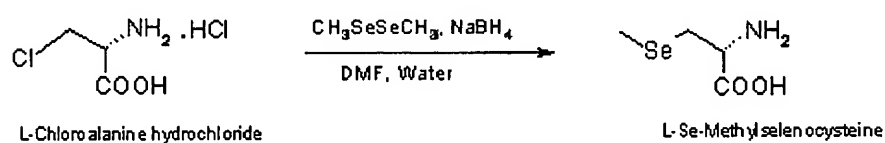
All the solvent was removed on a rotary evaporator at 90°C. To the residue obtained, 4ml of toluene was added and again evaporated to dryness. To the slightly gray colored solid, 75ml of isopropanol was added and stirred for 3 hours, cooled for 3 hours, filtered and dried in the oven for 2 hours at 60-70°C. Yield: 15g (76% from L-serine methyl ester hydrochloride); Melting point: 191-192°C (dec.) TLC analysis: n-Butanol: Water: Acetic acid (6:2:2).



L-Chloroalanine hydrochloride (10g) was dissolved in methanol and triethylamine was added drop wise at room temperature and pH of the reaction mixture was adjusted to 6. After stirring for 2 hours at room temperature, the precipitated L-chloroalanine was filtered, washed with 50ml methanol and dried. Yield: 7g (90%); Melting point: 164-165°C; TLC analysis: n-Butanol: Water: Acetic acid (6:2:2) $[\alpha]_D^{25}$: -16.28 (c 5.00, water); Chiral HPLC showed that the material obtained was only L-form.

Please replace paragraph [0023] presented in the amended specification dated January 8 2004 with the following original paragraph:

[0023] **Example 4 ~~Example 1~~ L-Se-Methyl Selenocysteine from L-Chloroalanine hydrochloride**



Dimethyldiselenide (50 g) in DMF (20 ml) was taken to get a clear solution. NaOH solution (24g in 100 ml water) was added under stirring. The mass was cooled to 5-10°C

and to this was added, portion-wise, solid sodium borohydride (6g) at $< 10^{\circ}\text{C}$. The reaction mixture was warmed to $40-45^{\circ}\text{C}$ and maintained for 2 hrs to get a clear colorless solution.

Please replace paragraph [0028] presented in the amended specification dated January 8 2004 with the following original paragraph:

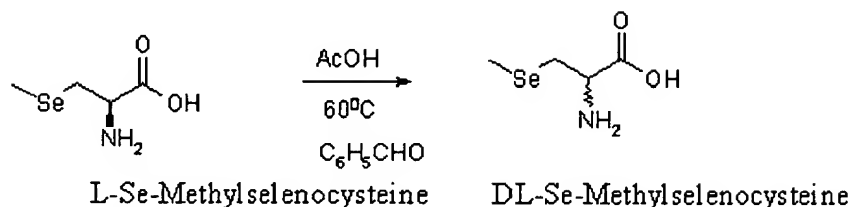
[0028] **Example 5 ~~Example-2~~ L-Se-Methyl Selenocysteine from L-Chloroalanine methyl ester hydrochloride:** Dimethyldiselenide (56 g) in DMF (25 ml) was taken to get a clear solution. NaOH solution (34g in 150 ml water) was added under stirring. The mass was cooled to $5-10^{\circ}\text{C}$ and to this was added, portionwise, solid sodium borohydride (7g) at $< 10^{\circ}\text{C}$ over a period of 1 hr. The reaction mixture was warmed to $40-45^{\circ}\text{C}$ and maintained for 2 hrs to get a clear colorless solution.

Please replace paragraph [0030] presented in the amended specification dated January 8 2004 with the following original paragraph:

[0030] **Example 6 ~~Example-3~~ L-Se-methylselenocysteine from L-chloroalanine hydrochloride** (using hypophosphorous acid to reduce dimethyldiselenide to methane selenol): In a reaction flask equipped with a stirrer and condenser dimethylformamide (25ml) and dimethyldiselenide (55 g) were taken under an atmosphere of nitrogen. To this solution was added slowly hypophosphorous acid (32% solution, 73g) over a period of 30 mts. The reaction mixture was slowly heated to 70°C and maintained for 2 hrs. The reaction mixture was cooled to 10°C and sodium hydroxide solution (20g in 100 ml water) was added slowly. The mixture was stirred for another 30 mts at that temperature and L-chloroalanine hydrochloride (25 g in 100 ml water) was added over a period of 1 hr. The reaction mixture was stirred for another 1 hr at RT and 1 hr at 40°C . After TLC indicated completion of the reaction, the reaction mixture was worked up as in Example 4. Yield : 10g

Please replace paragraph [0031] presented in the amended specification dated January 8 2004 with the following original paragraph:

[0031] **Example 7 ~~Example-4~~ DL-Se-Methyl selenocysteine**



A single-necked RB flask equipped with a magnetic stirring bar was charged with L-Methyl selenocysteine (0.5g), benzaldehyde(25 mg) and acetic acid (6 ml). The resulting suspension was heated to 60⁰C; After 15 minutes the reaction mixture became a clear solution. In another 20 minutes precipitation started. The mixture was stirred at this temperature for 2 hrs, then cooled to room temperature and filtered. The solid material was washed with ethanol thoroughly and dried in vacuo to afford 460 mg of white crystalline solid, DL-Se-MethylselenocysteineYield: 460mg, 92%; MP: 189-190⁰C. The chiral HPLC of this material (Figure 1) indicated only two peaks of equal areas attesting to its racemic nature; No other peaks were detected; the peak with lower RT corresponded to L-Methyl selenocysteine.

Please replace paragraph [0032] presented in the amended specification dated January 8 2004 with the following paragraph:

[0032] Convenient processes are described for the synthesis of L-Se-methylselenocysteine from chloroalanine derivatives. Chloroalanine itself is produced in a new method involving Serine methyl ester hydrochloride and thionyl chloride. The process is easily extendable to other selenium substituted amino acids. DL-Se-methylselenocysteine is easily obtained by a benzaldehyde-catalyzed racemization of L-Se-methylselenocysteine.